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## Physiological changes during natural ageing for seed vigour assessment of cowpea [*Vigna unguiculata* (L.) Walp] Genotypes

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### Abstract

Fifteen cowpea [*Vigna unguiculata* (L.) Walp.] genotypes namely DC-15, IPCW-20-210, IPCW-207, IPCW-21-247, IPCW-20-214, IPCW-21-51, KBC-9, IPCW-20-217, IPCW-20-123, IPCW-21-410, IPCW-7-3, RC-101, IPCW-3-20 and DCS-47-1 were classified as good, medium, and poor storers based on the physiological changes in the seeds over a six months of storage. The findings demonstrated a discernible decrease in seed quality indicators over the course of the storage period, with freshly collected seeds showing greater germination and vigour. In comparison to poor storer genotypes (IPCW-3-20, IPCW-3-15, and DCS-47-1), the good storer genotypes (DC-15, IPCW-20-210, IPCW-207, KBC-9, IPCW-21-247, IPCW-20-214, IPCW-21-51, and IPCW-20-217) maintained higher germination, seedling vigour indices, and lower electrical conductivity of seed leachate after six months of storage. Furthermore, a decrease in seed vigour and viability was linked to an increase in seed moisture content and electrical conductivity as a result of metabolic reserve depletion.

**Keywords:** Physiological, germination, electrical conductivity, viability, vigour, ageing

### Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is a tropical leguminous crop believed to have originated in Africa (Ng and Marechal, 1985) <sup>[23]</sup>. It is extensively cultivated across various regions, including Asia, Central and South America and Africa. As a staple food, cowpea is rich in nutrients, providing a substantial amount of protein (22-24%), carbohydrates (55-66%), dietary fibre, B-complex vitamins, essential minerals, iron (0.005%), calcium (0.08-0.11%), essential amino acids such as lysine, leucine and phenylalanine, along with a small quantity of lipids (1.3-1.5%).

Globally, it is grown on approximately 22.8 mha, yielding 21.29 mt with an average productivity of 637 kg/ha (Anon., 2022a) <sup>[5]</sup>. In India, the crop spans around 4 mha, producing 2.9 mt, with a productivity of 532 kg/ha. In Karnataka, the cultivated area is about 0.09 mha, with production and productivity recorded at 0.04 mt and 432 kg/ha, respectively (Anon., 2022b) <sup>[6]</sup>. It is also suitable for cultivation in rice fallow lands across various states such as Odisha, Madhya Pradesh, Chhattisgarh, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu.

As a biological living thing, the seed gradually loses quality. For maximum crop performance and appropriate seedling establishment, it is essential to maintain seed viability and vigour throughout storage (Pandita and Hosamani, 2025) <sup>[24]</sup>. Both genetic and environmental factors, such as the seed's innate genotypic potential, initial seed quality, moisture content, relative humidity, and temperature, have an impact on seed ageing. Seeds are especially vulnerable to quick physiological degradation in tropical and subtropical areas with high temperatures and humidity (McDonald, 1999) <sup>[22]</sup>.

Loss of vigour, poor seedling growth, and decreased germination are all consequences of physiological degradation during storage. Genetic heterogeneity among genotypes plays a major role in determining seed vigour and germination, two crucial markers of seed quality. The capacity of various cowpea genotypes to retain viability and vigour under storage conditions varies significantly, underscoring the significance of finding genotypes with higher storage

capability (Hosamani *et al.*, 2012; Siddaraj *et al.*, 2019; Sridevi *et al.*, 2025) [17, 28, 29]. Seeds typically reach their maximum vigour and viability at the point of physiological maturity (Hosamani *et al.*, 2012; Pandita and Hosamani, 2025) [17, 24], after which various physical and biochemical changes during storage gradually reduce their performance. Over time, the natural ageing process leads to a decline in seed vigour and viability, which can negatively impact seedling establishment and ultimately crop productivity. Hence, maintaining optimal storage conditions is essential to preserve seed quality, ensuring both genetic integrity and economic value over time (Rajjou *et al.*, 2007) [26].

Sustaining seed viability and attaining consistent crop establishment depend on maintaining appropriate storage conditions. The identification and selection of superior genotypes for breeding and hybridization programs is made easier by an awareness of genotypic diversity in seed storability and related physiological traits. In light of the aforementioned, the current study was conducted to examine the physiological changes in cowpea genotypes during natural ageing in order to select genotypes with higher storage potential and evaluate seed vigour.

### Materials and Methods

During *kharif* 2024-25, a lab experiment was carried out at the Department of Seed Science and Technology, College of Agriculture, Dharwad, UAS Dharwad, to examine physiological changes during natural ageing for the evaluation of 15 cowpea genotypes for seed vigour. The genotypes included DC-15, IPCW-20-210, IPCW-207, IPCW-21-247, IPCW-20-214, IPCW-21-51, KBC-9, IPCW-20-217, IPCW-20-123, IPCW-21-410, IPCW-7-3, RC-101, IPCW-3-20, IPCW-3-15 and DCS-47-1. After one cycle of multiplication, seeds were obtained from the ICAR-Indian Institute of Pulses Research (IIPR), Regional Research Center-cum-Off-season Nursery, Dharwad. Physiological changes were noticed in fresh seeds as well as after three and six months of storage. Harvested seeds were kept in cloth bags under ambient laboratory conditions.

Seed germination (%) according to ISTA (2021) [19] and seedling vigour index I and II, which were computed using the Abdul-Baki and Anderson (1973) [1] algorithm and given in numerical form, were among the physiological indicators used to assess vigour. With a few minor adjustments, the electrical conductivity (EC) ( $\mu\text{S}/\text{cm}/\text{g}$  seed fresh weight) of the seed leachate was calculated according to Agrawal and Dadlani (1992) [3]. According to ISTA (2021) [19], seed moisture content (%) was calculated using the hot air oven method and expressed on a fresh weight basis.

### Results

Seed germination of cowpea genotypes declined progressively with storage duration (Table 1). In freshly harvested seeds, among the 15 genotypes evaluated seven exhibited more than 90 per cent germination, while the remaining eight genotypes showed germination ranging from 66.75 to 89.25 per cent, with an overall mean of 87.32 per cent. The highest germination was recorded in KBC-9 (96%), followed by DC-15 (94.25%) and IPCW-20-217 (93%). In contrast, IPCW-3-20 exhibited the lowest germination (66.75%).

After three months of storage, germination declined across all genotypes with a pronounced reduction in poor storers. Among the good storer genotypes, DC-15 maintained the highest germination (88%), followed by IPCW-207 (85.75%) and KBC-9 (85.50%), whereas IPCW-20-214 recorded the lowest

germination (80%) within this group. In medium storers, IPCW-21-410 showed the highest germination (84%), while RC-101 recorded the lowest (60%). Poor storer genotypes exhibited a sharper decline, with germination ranging from 58 per cent in DCS-47-1 to 45 per cent in IPCW-3-15. The overall mean germination after three months of storage decreased to 74.47 per cent.

After six months of storage, germination was further reduced in all genotypes. Genotype DC-15 maintained the highest germination (86.50%), followed by IPCW-20-210 (81.25%) and IPCW-207 (79.50%), which were statistically on par. In contrast, a drastic reduction was observed in poor storers, with DCS-47-1 recording only 1.00 per cent germination, followed by IPCW-3-15 (13%) and IPCW-3-20 (21%). The overall mean germination declined further to 61.68 per cent.

The genotypes were divided into three groups based on the germination percentage following six months of storage. Superior seed storability was shown by good storers, which maintained more than 75% germination. Medium storers were defined as genotypes that shown germination between 50 and 75 per cent, whereas poor storers were defined as those that germinated less than 50 per cent. Good storers maintained sufficient seed viability, whereas medium and poor storer genotypes did not fulfill the Indian Minimum Seed Certification Standards (IMSCS, 2013) [18] of 75% germination for cowpea.

Seedling vigour indices (SVI-I and SVI-II) of cowpea genotypes declined progressively with storage duration (Table 1). In freshly harvested seeds, SVI-I varied considerably among the genotypes. The highest SVI-I was recorded in DC-47-1 (3359), followed closely by IPCW-3-15 (3352), IPCW-21-247 (3280), and IPCW-20-210 (3133), which were statistically on par. In contrast, IPCW-3-20 exhibited the lowest SVI-I (1637). The overall mean SVI-I for freshly harvested seeds was 2688. After three months of storage, SVI-I declined across all genotypes. The highest SVI-I was observed in IPCW-20-210 (3103), whereas IPCW-3-20 recorded the lowest value (1306). The overall mean decreased to 2198, indicating a notable reduction in seedling vigour. After six months of storage, further deterioration of vigour was evident in all genotypes. IPCW-21-247 (2782) and IPCW-20-210 (2746) maintained the highest SVI-I, followed by DC-15 (2420) and IPCW-20-214 (2419). The lowest SVI-I was observed in DCS-47-1 (30), highlighting severe vigour loss in certain genotypes. The overall mean SVI-I after six months was 1848. While good storers showed gradual reductions in vigour, medium and poor storers experienced a sharper decline with storage.

Seedling vigour index II displayed a similar trend. In freshly harvested seeds, the highest SVI-II was recorded in IPCW-20-210 (72777) and IPCW-3-15 (72060), while the lowest was observed in IPCW-7-3 (41578) and IPCW-21-51 (42313). The overall mean SVI-II was 55787. After three months, SVI-II declined markedly with IPCW-20-210 (62150) and IPCW-21-247 (61775) maintaining the highest values. The lowest values were recorded in IPCW-3-20 (33177) and IPCW-3-15 (34528) with an overall mean of 44265. After six months, further reductions were observed with the overall mean declining to 33435. Genotypes IPCW-20-210 (54967) and IPCW-21-247 (54052) retained the highest SVI-II, whereas DCS-47-1 recorded the lowest (583).

Across storability groups, good storers consistently maintained higher mean values of both SVI-I (2826, 2544 and 2412) and SVI-II (54609, 48922 and 42706) at fresh, three, and six months of storage, respectively than medium and poor storers. Medium storers exhibited intermediate values (2340, 2026, 1847 for SVI-

I and 51743, 41368, 33809 for SVI-II), whereas poor storers recorded the lowest vigour (2782, 1503, 346 for SVI-I and 64324, 35709, 8214 for SVI-II). This shows that seedling vigour gradually decreases with storage time, with the reduction being greatest in poor storers, moderate in medium storers, and least in good storers.

Seed moisture content of cowpea genotypes increased progressively with storage duration (Table 2). In freshly harvested seeds, the highest moisture content was recorded in IPCW-207 (10.87%), followed by IPCW-7-3 (10.82%), whereas IPCW-3-15 exhibited the lowest value (8.82%). The overall mean moisture content at harvest was 8.97 per cent. After three months of storage, seed moisture content increased in all genotypes. IPCW-207 maintained the highest moisture (11.85%), while IPCW-20-123 recorded the lowest (9.09%). The overall mean after three months was 9.26 per cent. Further increase in seed moisture content was observed after six months of storage. Genotype DCS-47-1 exhibited the highest moisture content (12.99%), whereas IPCW-20-123 recorded the lowest (10.85%). The overall mean moisture content after six months was 10.99 per cent. These results indicate a steady increase in seed moisture content with storage duration, reflecting typical moisture absorption under ambient storage conditions across genotypes.

The electrical conductivity (EC) of cowpea seed leachate increased progressively with storage duration, indicating deterioration of seed membrane integrity (Table 2). In freshly harvested seeds, EC varied among genotypes with the highest value observed in IPCW-20-123 (11.76  $\mu\text{S}/\text{cm}/\text{g}$ ) and the lowest in IPCW-20-210 (4.96  $\mu\text{S}/\text{cm}/\text{g}$ ). After three months of storage, EC increased across all genotypes. The highest EC was recorded in IPCW-3-15 (50.55  $\mu\text{S}/\text{cm}/\text{g}$ ), followed by RC-101 (50.31  $\mu\text{S}/\text{cm}/\text{g}$ ), whereas IPCW-20-214 (9.78  $\mu\text{S}/\text{cm}/\text{g}$ ) exhibited the lowest value. After six months of storage, a further increase in EC was observed with DCS-47-1 (88.51  $\mu\text{S}/\text{cm}/\text{g}$ ) showing the highest value and IPCW-20-214 (12.62  $\mu\text{S}/\text{cm}/\text{g}$ ) the lowest. Across storability classes, good storer genotypes (DC-15, IPCW-20-214, IPCW-20-210 and IPCW-207) consistently maintained lower EC values, indicating minimal membrane damage. In contrast, poor storers (IPCW-3-15, IPCW-3-20 and DCS-47-1) exhibited higher EC values, reflecting severe deterioration. The overall mean EC of all genotypes increased from 7.85  $\mu\text{S}/\text{cm}/\text{g}$  in fresh seeds to 20.29 and 30.35  $\mu\text{S}/\text{cm}/\text{g}$  after three and six months of storage, respectively.

## Discussion

Seed germination showed a progressive decline during storage, indicating gradual loss of viability across genotypes (Table 1). In freshly harvested seeds, most genotypes exhibited more than 90% germination with KBC-9, DC-15, and IPCW-20-217 performing superiorly. After three months of storage, germination decreased though good storers such as DC-15, IPCW-20-210 and IPCW-207 genotypes maintained higher values. After six months, a marked reduction was observed with poor storer genotypes DCS-47-1, IPCW-3-15 and IPCW-3-20 falling below the Indian Minimum Seed Certification Standards (IMSCS, 2013) [18], while good storers retained germination above 75 per cent. The overall mean reduced from 87.32 per cent in fresh seeds to 61.68 per cent after six months of storage. These findings show clear genotypic variations in storability, with good storers exhibiting superior viability and vigour retention. Similar patterns were seen in cowpea by Deshpande *et al.* (2011) [9], horse gram by Lokeshwari *et al.* (2024) [20] and mung bean by Sridevi *et al.* (2025) [29], who observed that the

rate of germination reduction varies with genetic constitution and storage capability of legume genotypes.

Seedling vigour indices declined significantly with increasing storage duration. Freshly harvested seeds recorded higher vigour, which progressively decreased after three and six months of ambient storage. Among the genotypes, DCS-47-1 showed the highest SVI-I (3359) in fresh seeds, while IPCW-3-20 had the lowest (1637). After six months of storage, IPCW-21-247 (2782) and IPCW-20-210 (2746) maintained higher SVI-I, whereas DCS-47-1 recorded the minimum (30). Similarly, SVI-II decreased from 72777 (IPCW-20-210) in fresh seeds to 583 (DCS-47-1) after six months. Good storers consistently exhibited higher mean values of both SVI-I and SVI-II at all storage intervals, followed by medium storers, while poor storers recorded the lowest vigour. The extent of decline was genotype dependent, with poor storers deteriorating faster. These results are consistent with those of Sridevi *et al.* (2025) [29] in mung bean, and Durga and Verma (2013) [11], Durga and Keshavulu (2015) [10] in horse gram, and Adebisi *et al.* (2008) [2] in sesame.

Seed moisture content increased steadily with advancing storage duration, indicating gradual absorption of atmospheric moisture under ambient conditions (Fig. 1). The overall mean rose from 8.97 per cent in freshly harvested seeds to 10.99 per cent after six months of storage. Elevated seed moisture content was associated with faster deterioration in poor storers such as DCS-47-1, IPCW-3-15 and IPCW-3-20, whereas good storers like IPCW-21-247, DC-15 and IPCW-20-214 retained lower moisture levels and superior quality. Maruthi (2006) [21] reported similar findings, highlighting the impact of ambient storage on seed hydration status and viability. The current findings are supported by the knowledge that high seed moisture content accelerates metabolic activity and reserve depletion (Bortey *et al.*, 2016; Gangambika *et al.*, 2022) [7, 12] and predisposes seeds to bruchid infestation (Aidbhavi *et al.*, 2023) [4].

Electrical conductivity of seed leachate also exhibited a distinct increasing trend with storage duration, reflecting progressive loss of membrane integrity (Fig. 1). The overall mean EC of seed leachate increased from 7.85  $\mu\text{S}/\text{cm}/\text{g}$  in fresh seeds to 30.35  $\mu\text{S}/\text{cm}/\text{g}$  after six months of storage. Poor storer genotypes such as DCS-47-1 (88.51  $\mu\text{S}/\text{cm}/\text{g}$ ), IPCW-3-15 (78.61  $\mu\text{S}/\text{cm}/\text{g}$ ) and IPCW-3-20 (78.31  $\mu\text{S}/\text{cm}/\text{g}$ ) recorded the highest EC values, whereas good storers such as IPCW-20-214 (12.62  $\mu\text{S}/\text{cm}/\text{g}$ ) and IPCW-21-51 (13.25  $\mu\text{S}/\text{cm}/\text{g}$ ) showed the lowest, indicating better membrane stability. Previous results in horse gram (Maruthi, 2006) [21], cotton (Goel and Sheoran, 2003) [13], soybean (Hosamani *et al.*, 2013a; Hosamani *et al.*, 2013b; Hosamani *et al.*, 2020; Panobianco and Vieira, 2007) [14, 16, 15, 25], and onion (Rao *et al.*, 2006) [27] are supported by the observed increase in EC caused by solute leakage and membrane degradation. Similar patterns were noted in mung bean by Chan and Mohammad Bin (2019) [18] and Sridevi *et al.* (2025) [29], who confirmed that lower seed quality and poor storability are correlated with higher EC values.

Poor storer genotypes (DCS-47-1, IPCW-3-20, and IPCW-3-20) showed significantly greater EC of seed leachate and a marked drop in seed germination and seedling vigour indices during storage. On the other hand, superior germination, vigour indices, and lower EC values were constantly maintained by good storers (DC-15, IPCW-20-210, IPCW-207, IPCW-21-247, IPCW-20-214, IPCW-21-51, KBC-9, and IPCW-20-217). With corresponding EC levels, medium storers (IPCW-20-123, IPCW-21-410, IPCW-7-3, and RC-101) demonstrated intermediate performance, maintaining moderate germination



and vigour. Additionally, the moisture content of seeds increased steadily throughout the course of storage with poor storers maintained relatively higher values, making them more vulnerable to quick deterioration. These findings unequivocally

showed that cowpea genotypes can be successfully categorized as good, medium, and poor storers based on their viability and vigour behaviour after six months of ambient storage.

**Table 1:** Seed germination behaviour of cowpea genotypes stored under ambient conditions

Genotypes		Seed germination (%)			Seedling vigour index I			Seedling vigour index II		
		Fresh seeds	3 MAS	6 MAS	Fresh seeds	3 MAS	6 MAS	Fresh seeds	3 MAS	6 MAS
Good storers	DC-15	94.25 (76.13)*	88.00 (69.73) *	86.50 (66.82) *	2912	2641	2420	53580	47643	45502
	IPCW-20-210	88.00 (69.73)	84.50 (66.82)	81.25 (67.41)	3133	3103	2746	72777	62150	54967
	IPCW-207	91.00 (72.54)	85.75 (67.82)	79.50 (63.08)	2548	2209	2127	46250	42217	38319
	IPCW-21-247	92.50 (74.11)	85.00 (67.21)	78.00 (62.03)	3280	2835	2782	70948	61775	54052
	IPCW-20-214	81.75 (64.71)	80.00 (63.43)	77.25 (61.51)	2515	2433	2419	47210	42965	36629
	IPCW-21-51	87.00 (68.87)	83.00 (64.90)	77.00 (61.34)	2834	2424	2337	42313	39365	35911
	KBC-9	96.00 (78.46)	85.50 (67.62)	76.50 (61.00)	2738	2352	2303	53770	52143	40929
	IPCW-20-217	93.00 (74.66)	82.50 (65.27)	76.00 (60.67)	2651	2353	2164	50024	43115	35341
Medium storers	Mean	90.44	84.28	79.00	2826	2544	2412	54609	48922	42706
	IPCW-20-123	83.75 (66.23)	76.75 (61.17)	70.25 (56.95)	2263	2034	2000	54832	45015	40141
	IPCW-21-410	88.25 (69.95)	84.00 (66.42)	67.00 (54.94)	2892	2624	2157	59815	53785	39209
	IPCW-7-3	75.00 (60.00)	70.25 (56.95)	64.25 (53.28)	1837	1738	1686	41578	36883	31741
	RC-101	89.25 (70.86)	60.00 (50.77)	54.75 (47.73)	2370	1708	1547	50750	29788	24143
Poor storers	Mean	84.06	72.75	64.06	2340	2026	1847	51743	41368	33809
	IPCW-3-20	66.75 (54.79)	48.75 (44.28)	21.00 (27.27)	1637	1306	552	51726	33177	14401
	IPCW-3-15	92.50 (74.11)	45.00 (42.13)	13.00 (21.13)	3352	1428	457	72060	34528	9658
	DCS-47-1	90.75 (72.29)	58.00 (48.88)	1.00 (5.74)	3359	1775	30	69185	39423	583
	Mean	83.33	50.58	11.67	2782	1503	346	64324	35709	8214
Overall mean		87.32	74.47	61.68	2688	2198	1848	55787	44265	33435
S.Em ( $\pm$ )		1.52	0.74	1.97	99.90	64.72	108.38	207.37	132.59	166.91
CD @ 1%		5.77	2.80	7.50	379.99	246.16	412.24	788.77	504.33	634.82
Significance		S	S	S	S	S	S	S	S	S

**Note:** MAS - Months after storage

S - Significance

\* Values in the parentheses are arc sin transformations

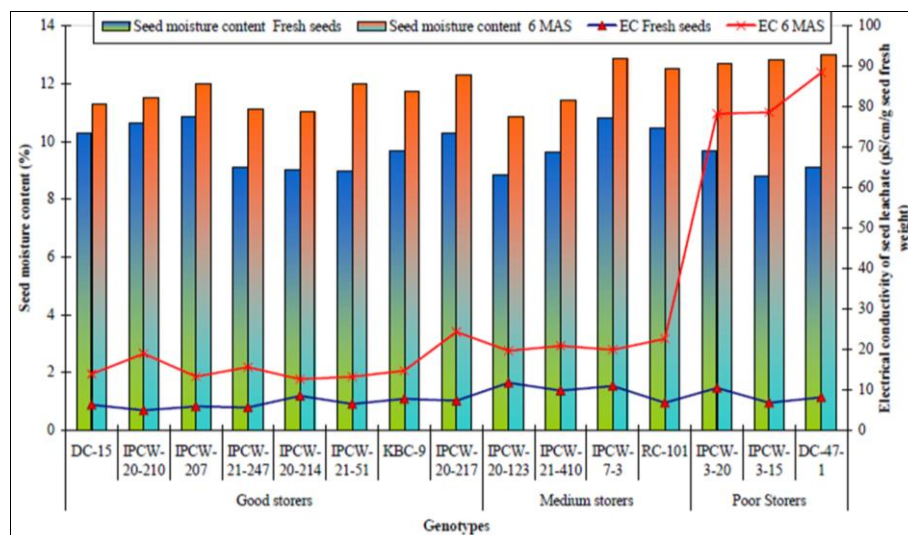
**Table 2:** Seed moisture content and electrical conductivity of seed leachate of cowpea genotypes stored under ambient conditions

Genotypes		Seed moisture content (%)			Electrical conductivity of seed leachate ( $\mu\text{S}/\text{cm}/\text{g}$ seed fresh weight)		
		Fresh seeds	3 MAS	6 MAS	Fresh seeds	3 MAS	6 MAS
Good storers	DC-15	10.29 (18.71)	10.56 (18.96)	11.32 (20.54)	6.28	10.03	13.89
	IPCW-20-210	10.65 (19.43)	10.75 (19.13)	11.51 (20.71)	4.96	13.24	18.97
	IPCW-207	10.87 (19.49)	11.85 (16.37)	11.98 (18.15)	5.89	11.80	13.28
	IPCW-21-247	9.09 (17.24)	9.35 (17.79)	11.11 (19.46)	5.64	13.31	15.63
	IPCW-20-214	9.03 (17.17)	9.29 (17.74)	11.05 (19.41)	8.52	9.78	12.62
	IPCW-21-51	8.98 (16.40)	10.25 (16.68)	12.01 (18.43)	6.51	10.39	13.25
	KBC-9	9.70 (17.86)	10.96 (17.42)	11.72 (19.11)	7.79	11.54	14.76
	IPCW-20-217	10.30 (18.71)	10.57 (18.96)	12.33 (20.55)	7.30	20.01	24.36
Medium storers	Mean	9.86	10.45	11.63	6.61	12.51	15.85
	IPCW-20-123	8.83 (16.21)	9.09 (17.52)	10.85 (19.21)	11.76	15.91	19.64
	IPCW-21-410	9.64 (18.20)	10.39 (16.96)	11.42 (18.25)	9.84	12.92	20.88
	IPCW-7-3	10.82 (19.20)	11.09 (19.45)	12.88 (21.10)	10.97	16.32	19.91
	RC-101	10.48 (18.89)	10.75 (19.13)	12.51 (20.71)	6.81	50.31	22.65
Poor storers	Mean	9.94	10.33	11.92	9.85	23.87	20.77
	IPCW-3-20	9.70 (17.86)	10.96 (17.42)	12.72 (19.11)	10.49	14.66	78.31
	IPCW-3-15	8.82 (16.18)	11.09 (19.45)	12.85 (21.00)	6.81	50.55	78.65
	DCS-47-1	9.10 (17.30)	10.76 (14.61)	12.99 (21.56)	8.16	43.61	88.51
	Mean	9.21	10.94	12.85	8.49	36.27	81.82
Overall Mean		8.97	9.26	10.99	7.85	20.29	30.35
S.Em ( $\pm$ )		0.51	0.54	0.44	0.16	1.22	0.75
CD @ 1%		2.14	2.24	1.82	0.61	4.74	2.90
Significance		S	S	S	S	S	S

**Note:** MAS - Months after storage

S - Significance

\* Values in the parentheses are arc sin transformation



**Fig 1:** Seed moisture content and electrical conductivity of seed leachate of cowpea genotypes stored under ambient conditions

## Conclusion

Increased electrical conductivity of the seed leachate indicates membrane breakdown, and prolonged seed storage dramatically lowers germination and seedling vigour. Certain cowpea types preserved higher storability due to genotypic variations. These findings emphasized that to choose suitable genotypes and implement the best storage techniques in order to maintain seed quality, guarantee consistent seedling establishment, and improve sustainable crop output.

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